

THE INFLUENCE OF HYPERTONIC AND HYPOTONIC SEA WATER ON THE ARTIFICIAL ACTIVA- TION OF STARFISH EGGS

RALPH S. LILLIE

*(From the Department of Physiology, University of Chicago, and
the Marine Biological Laboratory, Woods Hole, Massachusetts)*

In the previous paper (2) the general conditions of artificial activation in the starfish egg were summarized briefly as follows: (a) a non-toxic penetrating acid (*e.g.* a fatty acid) acting alone, either in the presence or absence of oxygen, activates the egg completely and at a rapid rate which is closely proportional to the cH of the acid solution¹; (b) hypertonic sea water may also cause complete activation but at a much slower rate and only in the presence of oxygen.

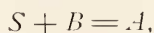
The important fact is that either of these two quite different changes of condition may produce the same physiological effect; but apparently they do so by inducing or promoting within the egg biochemical reactions of dissimilar kinds.² The hypothesis was proposed that the acid acts by catalyzing the hydrolytic decomposition of some ester-like compound (possibly phosphagen) normally present in the egg, and that the reaction which directly determines activation consists in the chemical union of a product of this hydrolysis with some other compound also present or available in the egg. This second compound appears to be formed (or its quantity increased) under the influence of hypertonic sea water in the presence of oxygen, the presumption being that the abstraction of water promotes its dehydrolytic synthesis from certain pre-

¹ Heat activation is regarded as a special case of acid activation.

² Certain other characteristic differences in the physiological effect of the two treatments are in harmony with this conclusion. Thus, if the eggs are exposed briefly to heat (32°–35°) or acid (*e.g.*, .004M butyric acid in sea water) within a few minutes (up to ten) after removal from the starfish, they not only show no activation but remain permanently immature with germinal vesicle intact (4). Hypertonic sea water, on the contrary, activates the eggs in a typical manner when the exposures are begun during this period. The immediate effect produced by the hypertonic sea water is apparently independent of the breakdown of the germinal vesicle; while the acid is quite without activating effect until this change has occurred, after which the egg is readily activated by both heat and acid. Apparently the substance which reacts with the acid is either absent or unavailable until the germinal vesicle has broken down.

cursors. The combination of the two compounds produces a third compound, the activating substance, whose accumulation renders the egg capable of automatic development.

According to this hypothesis the critical change determining activation is the chemical union of the hydrolytic product, which we designate as B , with the synthetic product, called S , to form the activating substance, A . This reaction (activation reaction) would then be represented by the equation



and its velocity by the equation

$$I' = K C_s C_b,$$

where K is the velocity constant of the reaction, and C_s , C_b the concentrations of S and B respectively.

B is regarded as being freed by the activating acid at a rate proportional (through a certain range) to the cH ; it combines as rapidly as it is formed with S ; hence the proportionality between the cH and the rate of activation. In normal untreated eggs the concentration of S (*i.e.*, C_s) is assumed to be a constant, fixed by the inherited constitution of the egg. With C_s thus constant, the rate of activation should be proportional to the concentration of the activating solution of acid, as we find (3). If, however, we vary C_s , as by previously treating the eggs with hypertonic sea water, the rate of activation in a constant solution of acid should vary correspondingly; under such circumstances this rate, according to the formula, would be proportional to C_s .

Experiment shows that unfertilized eggs treated for a brief period (5–40 minutes) with hypertonic sea water and then exposed to acid show the usual course of acid activation, but at an increased rate (Table I). This characteristic effect may be described by saying that the eggs are *sensitized* to the activating influence of the acid.³ According to our hypothesis the effect follows from the dehydrolytic synthesis of additional S (called also the sensitizing substance). In this synthesis a number of simple molecules are assumed to interact to form a molecule of the more complex compound S ; in the successive steps of any such synthesis the molecules are to be conceived as combining successively in pairs; and the rate of the total synthesis will be determined by the rate of interaction of the pair of molecules which combine most slowly.⁴

³ The analogy with the sensitization of photoreceptors to light during the process of dark-adaptation, which follows a very similar time course (6, 7), is pointed out below.

⁴ The relation of oxygen to hypertonic activation suggests that the combination of molecular oxygen with some other compound may be the controlling reaction.

The course of hypertonic sensitization would then be expected to follow the curve of a bimolecular reaction. In the following experiments the rate of activation by a constant solution of butyric acid, .004M in sea water, has been determined after exposing the eggs for varying periods to hypertonic sea water of constant composition.

ACTION OF HYPERTONIC SEA WATER FOLLOWED BY ACID

A typical experiment is summarized in Table I. In all experiments of this kind the eggs were placed, shortly after the breakdown of the germinal vesicle, in hypertonic sea water of a constant composition (100 volumes sea water *plus* 20 volumes 2.5 M NaCl). After varying intervals, usually 10, 20, 30 and 40 minutes, portions were transferred to normal sea water, where they remained 10 or 15 minutes; they were then transferred to .004 M butyric acid solution in sea water. From this solution portions were returned to normal sea water at regular intervals (usually of 1 minute between 1 and 10 minutes). The approx-

TABLE I

July 7, 1932. Eggs from several animals were removed at 3:40 and placed in hypertonic sea water at 4:10. Temperature of solutions and sea water 22°. In the fertilized control practically all eggs developed normally.

In hypertonic sea water (22°)	Interval in normal sea water	Percentages of eggs forming blastulae after exposure to .004 M butyric acid (22°) for the times indicated (min.)								
		(Control: hyp. sea water alone)	2	3	4	5	6	7	8	9
<i>min.</i>	<i>min.</i>									
A. 0*		0	pr. 0	15-20	40-50	70-80	80-90	70-80	50-60	
B. 10	10	0	pr. 0	ca. 1	30-40	ca. 90	70-80	ca. 50	30-35	ca. 10
C. 20	12	0	<1	ca. 20	55-65	60-70	ca. 50	ca. 5	0	0
D. 30	12	pr. 0	ca. 1	25-30	70-75	50-60	30-40	3-5	0	0
E. 40	12	<1	5-10	40-50	65-70	ca. 50	15-20	ca. 5	0	0

* Control: acid alone.

imate percentages of eggs developing to blastulae were later determined. Controls of eggs exposed to hypertonic sea water without acid treatment, and to butyric acid without hypertonic treatment, were always kept.

In this experiment the series treated with butyric acid alone (*A*) shows the typical increase in the proportion of favorably developing eggs with increasing time of exposure up to a definite optimum at about 7 minutes, after which there is a decline. With the eggs treated previously with hypertonic sea water a similar time curve of activation is

seen in each of the series *B* to *E*, but the effective exposures (as measured by the formation of blastulæ) are in all cases briefer, and the more so the longer the treatment with hypertonic sea water. The optima are respectively *A*, 7–8 minutes; *B*, 5 minutes; *C*, 4–5 minutes; *D*, *ca.* 4 minutes; *E*, *ca.* 4 minutes.

In ten experiments of this kind in June and early July a similar result was found. In two of these (June 10 and June 11, 1931) the temperature of the solutions was 18° and the optimal exposures to the acid alone were longer (*ca.* 20 minutes). The other eight experiments are summarized in Table II; these were carried out under closely similar conditions; the temperature of the hypertonic sea water and butyric acid solution was 20°–22°, and the optimal exposures to the acid alone varied between 7 and 9 minutes. The table gives the results of exposures to butyric acid after treatment with hypertonic sea water (100 + 20) for periods varying from 10 to 40 minutes. The single experiments are in good agreement, and the averages show a regular decrease of activation-time with increase in the duration of the hypertonic treatment. Up to 40 minutes few or no eggs develop to blastulæ as a result of the hypertonic treatment alone; but an exposure of 60 minutes to hypertonic sea water alone usually activates a considerable portion of eggs, so that the sensitizing effect then becomes difficult to estimate. In the experiments of June 17 and June 18 eggs exposed to hypertonic sea water for 60 minutes formed a considerable proportion of blastulæ without further treatment (*ca.* 10 per cent), and 50 per cent or more blastulæ with additional exposures of 1 and 2 minutes to butyric acid.

The data show that the action of hypertonic sea water in shortening the effective exposures to butyric acid is most rapid during the earlier part of the immersion and falls off regularly as time advances. In Fig. 1 the average optimal exposures to acid (optimal activation-times) are plotted against the times in hypertonic sea water. The curve drawn is the theoretical bimolecular isotherm, the assumptions being (1) that the rate of the activation-process (the reciprocal of the activation-time) is proportional to the concentration of *S*, and (2) that the rate of formation of *S* is controlled by the interaction of two precursor molecules of equal concentration ($P + Q = S$). The simple bimolecular formula is used,

$$\frac{1}{t} \cdot \frac{x}{a(a-x)} = K;$$

a represents the initial concentration of *P* or *Q*, and *x* the concentration of *S* formed by the time *t*. The initial concentration, *a*, is equal to the difference between the concentration of *S* when the reaction is complete,

TABLE II

Optimal exposures (minutes) to butyric acid solution (.004 M in sea water at 20–22°) and percentages of eggs forming blastulae (in brackets) after previous treatment with hypertonic sea water for varying periods.

Experiment no.	Date	Durations of previous exposure (minutes) to hypertonic sea water (20°-22°)						
		0 (control)	10	15	20	30	40	60
1.	June 17	9 (75-80)			5 (30-35)		4 (30-40)	1-2 (ca. 50)
2.	June 18	7 (80-90)			5-6 (ca. 50)		4 (ca. 50)	1-2 (ca. 50)
3.	June 29	7 (ca. 50)			4 (20-30)	4 (20-30)		
4.	June 30	7-8 (65-75)	5 (35-40)		5 (40-50)	5 (35-45)	4 (35-45)	
5.	July 6	8 (70-80)	6 (50-60)		4 (30-35)	3 (35-40)	2-3 (35-45)	
6.	July 7	7 (80-90)	5 (55-65)		4-5 (55-70)	4 (70-75)	4 (65-70)	
7.	July 8	7 (75-85)	5 (80-90)	5 (50-60)		4 (ca. 50)	3 (ca. 40)	
8.	July 11	8 (70-80)	5 (60-70)		5 (ca. 50)	3-4 (30-35)	3 (30-35)	
Averages		7.5 min.	5.2 min.	5 min.	4.7 min.	3.9 min.	3.5 min.	

and its concentration at the beginning of the reaction (*i.e.*, before any hypertonic treatment); this difference is regarded as proportional to the difference between the optimal activation-times at these two periods. $a-x$ is the concentration of S yet to be formed at any stage in the reaction; *i.e.*, when the latter is complete $a-x$ becomes zero. S is steadily being formed during the immersion in hypertonic sea water but at a progressively decreasing rate. The time required for complete transformation of the precursors into S is estimated by the extrapolation of the curve to its asymptote or final level. This time is estimated at about 90 minutes, and the limiting value of the activation-time as 2 minutes. The calculated curve is drawn on this basis (see Table III), and the position of the points representing the observations shows a good correspondence with the theory.⁵

TABLE III

The final concentration of S is regarded as that corresponding to an optimal activation-time of 2 minutes. The value of a is then $7.5 - 2.0 = 5.5$. The additional concentrations of S formed after the times t are regarded as proportional to the decreases in the optimal activation-times (column 3).

Time in hypertonic sea water (t)	Optimal activation times	Additional S formed at time t (x)	P or Q at time t ($a-x$)	K	Activation-times calculated from average value of K
<i>min.</i>	<i>min.</i>				<i>min.</i>
0	7.5	0			
10	5.2	2.3	3.2	0.013	5.36
20	4.7	2.8	2.7	0.010	4.43—
30	3.9	3.6	1.9	0.0115	3.88
40	3.5	4.0	1.5	0.012	3.56
				(average 0.0115)	

It is interesting to note that the curve resembles in its general character the curves representing the course of sensitization to light during the process of dark-adaptation, as recently investigated by Hecht. He

⁵ The estimated activation-time of 2 minutes corresponding to the maximal concentration of S cannot be determined directly, for the reason that after sufficient exposure to hypertonic sea water many eggs develop completely without any acid treatment. This has already occurred at 60 minutes in the two experiments of June 17 and 18 (Table II). The optimal exposures to hypertonic sea water alone at 20° are between 75 minutes and 2 hours; this would indicate that maximal sensitization has then been reached. On the average there is not much difference in the effects of exposure between 75 and 120 minutes. Runnström finds that acid is formed in the sea-urchin egg after prolonged exposure to hypertonic sea water (6); such an effect would account for the formation of the hydrolysate B required for the assumed reaction $S + B = A$, and would also explain why after 60 minutes in hypertonic sea water the effect of the butyric acid solution is greater than would otherwise be expected.

regards dark-adaptation as resulting from the accumulation of a light-sensitive compound formed by a bimolecular (*i.e.*, bimolecularly-controlled) reaction, and he has shown that many forms of dark-adaptation follow a curve of this type (7). Hartline has recently shown the same for the eye of *Limulus* (8). Hecht's curve of the light-sensitization of *Pholas* during $2\frac{1}{2}$ hours in the dark (9) may be compared with Fig. 1.

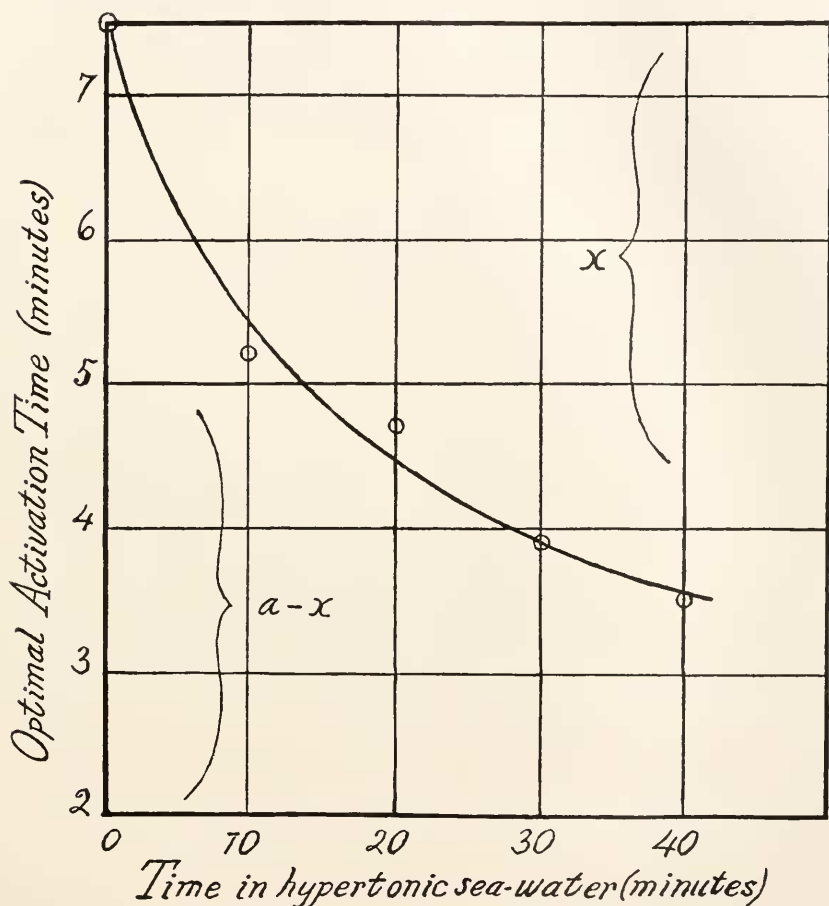


FIG. 1. Ordinates: average optimal activation-times (minutes of exposure to .004 M butyric acid in sea water). Abscissæ: previous exposures to hypertonic sea water (minutes). Temperature 20° - 22° (see Table II).

A constant light-intensity (analogous to the constant acid-concentration used in the present experiments) was used for stimulation, and the reaction-time ($\equiv$ activation-time) was found to decrease progressively to a minimum, in a manner resembling closely that just described.

The fact that the sensitizing effect of hypertonic sea water appears so soon after immersion and that the curve shows so steep a descent during the first few minutes, indicates that the loss of water from the site of the sensitizing reaction is rapid and reaches equilibrium almost immediately. Localization of the reaction in the most superficial layer of the egg cortex would thus be indicated, possibly the layer immediately beneath the outer pellicle; the latter is apparently scleroprotein in nature, and is separated on activation as the fertilization membrane (10). This would imply that a more internal layer controls the osmotic loss of water from the egg as a whole, a process requiring apparently about 10 minutes to reach equilibrium, as indicated by the curve of shrinkage in sea water made hypertonic with glycol (11, 12).

BUTYRIC ACID FOLLOWED BY HYPERTONIC SEA WATER

When a brief exposure to butyric acid, sufficient for partial but not for complete activation, precedes the hypertonic treatment, the results are less regular than with the reverse order. The effective exposures to hypertonic sea water are shortened, but the increase in rate of response to this agent (as measured by the decrease in effective exposures) shows no very definite relation to the time in butyric acid solution. When the exposures to acid are sufficient for complete or almost complete activation, the subsequent treatment with hypertonic sea water is ineffective or injurious; while with longer than optimal exposures it is definitely injurious.

These effects are illustrated by the two series summarized in Tables IV and V. The eggs were placed, shortly after the breakdown of the germinal vesicle (30–35 minutes after removal from the starfish), in .004 M butyric acid in sea water for the times indicated; they were then returned to normal sea water, and after 15 or 20 minutes they were exposed to hypertonic sea water. The percentages forming blastulæ and gastrulæ are given as before; the results of single treatments with acid or hypertonic sea water are also given. Both lots of eggs were normal and responded normally to sperm fertilization. Table IV gives the results observed with briefer exposures to acid, and Table V those with longer exposures ranging from sub-optimal (6 minutes) to supra-optimal (12 minutes).

In fourteen experiments of this kind during the summers of 1931–32 the results were in general similar but with considerable variation in detail. The previous treatment with acid appears to disturb the regularity of the response to hypertonic sea water, possibly because of changes in the osmotic properties of the surface layer; and regular quantitative relations of the kind observed with the reverse order of treat-

ment are difficult to discern. The effective exposures to hypertonic sea water are, however, almost invariably shortened by brief treatment with butyric acid; this effect may be attributed to the formation of the acid-product, *B*.

TABLE IV

Experiment of June 26, 1931. Concentrations of hypertonic sea water and butyric acid solution the same as before; temperature of both *ca.* 20°.

Time in acid solution	Durations of exposure to hypertonic sea water (minutes) and percentages of resulting blastulae								
	(Control: acid alone)	10	20	30	40	50	60	75	90
<i>min.</i>									
A. 0*		0	0	<1	20-25	30-40	50-60	60-70	65-75
B. 1	0	<i>ca.</i> 1	20-25	30-40	40-45	<i>ca.</i> 50	40-50	<i>ca.</i> 50	
C. 2	0	<i>ca.</i> 1	<i>ca.</i> 10	40-50	<i>ca.</i> 50	50-60	<i>ca.</i> 50	30-40	
D. 4	0	<i>ca.</i> 1	<i>ca.</i> 1	10-15	40-50	<i>ca.</i> 50	50+	50-60	
E. 6	15-20	<i>ca.</i> 1	<i>ca.</i> 1	<i>ca.</i> 1	<i>ca.</i> 1	<i>ca.</i> 1	3-5	3-5	

* Control: hypertonic sea water alone.

TABLE V

Experiment of June 29, 1932. Similar conditions. Temperature 21-22°.

Time in acid solution	Exposures to hypertonic sea water (minutes) and percentages of blastulae							
	(Control: acid alone)	15	30	45	60	75	90	105
<i>min.</i>								
A. 0*		<1	2-3	<i>ca.</i> 50	<i>ca.</i> 90	85-90	75-85	60-70
B. 6	60-65	60-70	70-80	60-70	65-75	60-65		
C. 8	90+	50-60	50-60	30-40	30-40	25-35		
D. 10	30-40	<i>ca.</i> 5	<i>ca.</i> 1	<1	<1	<1		
E. 12	<1	<1	<1	0	0	0		

* Control: hypertonic sea water alone.

HYPOTONIC SEA WATER FOLLOWED BY BUTYRIC ACID

Theoretically we should expect that if hypertonic sea water acts by reducing the concentration of water at the site of the activation reaction, thus shifting the equilibrium in the direction of the further dehydrolytic synthesis of the sensitizing compound, *S*, the action of dilute sea water would be in the opposite direction; *i.e.*, previous exposure to hypotonic sea water should lengthen the activation-time in butyric acid solution.

Experiments were performed similar to those already described, but with hypotonic substituted for hypertonic sea water. The following dilutions of sea water were used: 15, 20, 25, 30, 35 and 40 volumes distilled water in 100 volumes of the mixture (*i.e.*, 85, 80, 75 etc. volumes per cent sea water). The eggs were placed in the hypotonic medium for periods of 15 to 30 minutes (*ca.* 20°), returned to normal sea water for 10 or 15 minutes, and then exposed to .004 M butyric acid in normal sea water.

Eggs treated with dilute sea water and then returned to normal sea water without further treatment show no membrane-formation or other signs of activation. The osmotic intake of water has thus by itself no evident activating action. Previous treatment with slightly dilute sea water (80 to 85 volumes per cent) has in some cases been followed by retardation of acid-activation, but the results have not been uniform. With the higher dilutions (75–60 volumes per cent sea water) the reverse effect was usually seen. This apparent acceleration of acid-activation by the more dilute sea water appears to be a summation effect. The conditions suggest that in the more dilute solutions the hypotonic distension has in itself some activating (acid-producing) effect which may compensate or mask the influence of dilution on the S-producing reaction.

INFLUENCE OF HYPERTONIC AND HYPOTONIC SEA WATER ON ACTIVATION BY WARM SEA WATER (32°)

During the course of maturation starfish eggs lose their responsiveness to heat-activation sooner than to acid-activation. After the separation of the first polar body (60–65 minutes after removal in sea water at 20°), eggs exposed to sea water at 32° for the optimal time give few if any blastulæ, while the response to butyric acid remains normal for some time longer. After the second polar division (90–100 minutes after removal), the response to acid declines rapidly. At 2 hours few if any eggs form blastulæ.

The time during which experiments with combined heat and hypertonic treatments can be carried out with fully responsive eggs is thus somewhat abbreviated. Nevertheless, the general type of result is the same as with acid-activation. In eggs treated previously with hypertonic sea water the response to heat is definitely accelerated. This has been established in a large number of experiments, the details of which need not be given. The results have shown somewhat less quantitative regularity than in the case of acid-activation; this difference may be attributed partly to the greater difficulty of controlling the conditions

and partly to variations in the acid-producing reaction within the egg.⁶

The following experiment (July 6, 1933) is typical (Table VI). Eggs were exposed to hypertonic sea water (beginning 30 minutes after removal) for (*B*) 15 minutes and (*D*) 38 minutes, returned to sea water (20°) for a short time, and then exposed to the warm sea water (32°). The controls (*A* and *C*) were treated (at the same interval after removal as *B* and *D*) with warm sea water alone.

With all times of exposure to 32° (from 2 minutes on) activation was more rapid in the eggs treated previously with hypertonic sea water. The eggs in Experiment *C* were exposed after the first polar division and show only slight response; in Experiment *D* maturation has been delayed during the stay in hypertonic sea water and responsiveness remains good at the same interval after removal. Hypertonic sea water has been found uniformly to retard the loss of response to heat-activation, besides decreasing the effective times of exposure.

TABLE VI

Treatment of eggs	Result
<i>A.</i> 32° alone in sea water, beginning 51 min. after removal.....	Optimum at 6 min.: 60-65% blastulæ
<i>B.</i> Hypertonic sea water for 15 min.; normal sea water 12 min.; then 32° in sea water, beginning 52 min. after removal..	Optimum at 4-5 min.: 65-75% blastulæ
<i>C.</i> 32° alone, beginning 80 min. after removal.....	Optimum at <i>ca.</i> 5 min.; few blastulæ (<i>ca.</i> 5%)
<i>D.</i> Hypertonic sea water 38 min.; sea water 8 min.; 32° in sea water, beginning 81 min. after removal.....	Optimum at 4 min.; many blastulæ (<i>ca.</i> 90%)

Experiments with hypotonic sea water have so far been few, and the influence on activation times was not constant. In this case also the loss of responsiveness is delayed during immersion in the dilute medium.

HYPERTONIC SEA WATER AND ACID ACTING SIMULTANEOUSLY

In the experiments so far described, where fatty acid and hypertonic sea water were used in combination, the two treatments were applied successively, with a brief interval in normal sea water. When the treat-

⁶ If we regard heat-activation as a special case of acid-activation, it would seem that some reactant or catalyst in the acid-producing reaction within the egg is lost or destroyed near the time of the first polar division. The other substances required for activation by acid (whether the acid is formed within the egg or enters from without) appear to remain unaltered for some time longer, at least until the second polar division, after which the response to all types of activation, including sperm-fertilization, rapidly declines.

ments are applied simultaneously, *i.e.*, when the eggs are exposed to hypertonic sea water containing .004 M butyric acid, a somewhat unexpected result is obtained. The activating action of the acid is markedly retarded, and the period during which this action is exhibited is correspondingly prolonged.

The following experiment will illustrate (June 22, 1933). Eggs of the same lot were treated simultaneously during the prematurational period with .004 M butyric acid dissolved (*A*) in normal sea water and (*B*) in hypertonic sea water, and then returned to sea water. Table VII gives the durations of exposure and the approximate percentages of eggs forming blastulae.

Eight experiments of this kind were carried out during June 1933, and in all a similar effect was shown, with some variation in degree. The delay in activation appears to be correlated with a delay in the structural change associated with activation. In all experiments mem-

TABLE VII

Times of exposure (minutes at 20.5°) and percentages of blastulae

Solution used	2	4	6	8	10	12	14	16	18	20	22	24
A. .004 m. butyric acid in normal sea water.....	ca. 1	ca. 5	50	80-90	ca. 80	20-25	<5	0				
B. .004 m. butyric acid in hypertonic sea water.....	ca. 1	ca. 1	<5	15-20	30-40	70-80	70-80	60-70	ca. 60	20	10	0

brane-formation was prevented or retarded, the range of effective exposures was lengthened, and the breakdown following supra-optimal exposures was delayed. In one case 50 per cent of eggs formed blastulae after an exposure of 24 minutes.

There is much evidence that a structural change in the surface-layer or "cortical zone" of the egg is a constant factor in both normal and artificial activation in many species; this evidence is seen in membrane-formation in echinid eggs, increase of permeability to water and dissolved substances, visible breakdown of the cortical layer in annelid eggs, and similar effects. Conversely, agents which alter the structure of the surface-layer, such as cytolytic compounds, mechanical agitation, and strong electric currents, have an activating effect.⁷ The retarding

⁷ The fact that the progress of the activation-reaction in warm sea water has such a high temperature-coefficient (1), and that the reaction may be immediately arrested at any stage by returning the eggs to sea water, suggests again a dependence of activation on some readily reversible structural change. Activation

influence of hypertonic sea water on acid-activation, shown in the experiments just described, may be interpreted as an expression of increased structural coherence or density resulting from the partial dehydration of the surface-layer. To the delay in the structural change corresponds the delay in the primary chemical reaction of activation. In the terms of our general hypothesis, the rate of union of the reactants *S* and *B* may be regarded as determined in part by the structural conditions (*e.g.*, permeability conditions) controlling the rates of diffusion at the site of the activation-reaction, as well as by the conditions of concentration already considered. Such a view would account both for the retardation of the reaction and for its being spread out over a longer period of time.

NATURE OF THE CHEMICAL REACTIONS OF ACTIVATION

The special nature of the chemical reactions underlying activation is still obscure. In the former paper (2) it was pointed out that certain conditions of acid-activation (such as independence of oxygen-consumption) indicate a hydrolysis or other type of non-oxidative splitting process as the most probable type of primary reaction, and from the analogy with other cells (muscle, yeast, nerve) the splitting of a phosphagen ester was suggested. An attempt to test this hypothesis was made in the summer of 1932, by the analytical estimation of the free phosphate in the eggs before and after fertilization; but the analyses⁸ (somewhat few in number) gave no indication of any change. A further corollary of the phosphagen hypothesis is that the other possible hydrolytic products might be concerned in the reaction-sequence of activation; but experiments in which eggs were warmed at 32° (1 to 9 minutes) in sea water containing the phosphagen-forming bases, creatin, arginin, and guanidin (0.1 to 0.4 per cent creatin hydrate, and hydrochlorates of the other bases in similar concentrations) also gave essentially negative results. If such compounds penetrate the egg in the warm sea water, as it seems reasonable to suppose—since (*e.g.*) hydrochloric acid in low concentration gives evidence of penetrating under these conditions (2)—they are without influence in the rate of the activation-reaction. Up to the present, at least, the phosphagen hypothesis has received no support from the experiments which it has suggested.

A similar uncertainty exists with regard to the nature of the acid arrested in this way can be renewed and carried to completion by a second exposure to heat, or by exposure to acid or hypertonic sea water (1).

⁸ Carried out by Dr. Pei-Sung Tang, to whom my best thanks are due. Runnström also has been unable to find any change in the free phosphate of sea-urchin eggs after fertilization (6).



which we have inferred—from the close resemblance between heat and acid-activation—to be formed within the egg during the exposure to warm sea water. If heat-activation is a form of acid-activation, the most obvious supposition would be that lactic acid is formed (within the egg-cortex) by glycolysis, very much as in the heat rigor of muscle. Increase in lactic acid following fertilization has recently been observed in sea-urchin eggs by Perlzweig and Barron (13). I have found, however, that iodoacetic acid, which blocks glycolysis in a large variety of cells, does not interfere appreciably with heat-activation in starfish eggs. In three successive experiments in which eggs (from three different lots) were warmed to 32° in sea water containing 0.02 per cent Na-iodoacetate the course of heat-activation was entirely normal, 60 per cent or more of the eggs forming blastulæ with exposures of 4 to 6 minutes. The only noticeable difference from the control (32° in normal sea water) was a slight shortening of the activation-time in the presence of iodoacetate and the production of a somewhat larger proportion of blastulæ. This result seems quite inconsistent with the glycolysis hypothesis of heat-activation, unless we assume absence of penetration, or that iodoacetate is without influence on the acid-forming reaction in these eggs.⁹ The possibility may be considered that some acid other than lactic, *e.g.*, pyruvic, is responsible for heat-activation. Further experimental and chemical work is required.

SUMMARY

Brief treatment of unfertilized starfish eggs with hypertonic sea water increases the rate of activation by subsequent exposure to butyric acid. This effect may be described as a sensitization to the activating influence of the acid. The curve representing the relation between the duration of the hypertonic treatment and the rate of acid-activation resembles the bimolecular reaction isotherm.

In general the experimental facts of acid-activation (and of heat-activation) are consistent with the following simplified schema. Activation is the physiological result of the accumulation of a reaction product, called (provisionally) "the activating substance" (substance *A*) which is formed by the chemical union of two chief compounds. One

⁹ Sodium fluoride also, in experiments with .002, .004, .008 and .01 per cent solutions in sea water, was found not to interfere with heat-activation. In one experiment 90 per cent of eggs formed blastulæ after 6 minutes in sea water at 32° containing .01 per cent NaF.

Dr. E. S. Guzman Barron informs me that he has found iodoacetate to have no depressant action but, if anything, a slight promoting action upon oxygen-consumption in *Arbacia* eggs.

of these, called (after the analogy with light-sensitization) the "sensitizing substance" (substance *S*), is already present in the egg; the other, the "acid-product" (substance *B*), is set free (*e.g.*, by hydrolysis) under the influence of the activating acid. The equation $S + B \rightleftharpoons A$ symbolizes this hypothetical activation-reaction.

Treatment with hypertonic sea water increases the concentration of *S* at the site of the activation-reaction, through the abstraction of water and promotion of dehydrolytic synthesis. The resulting increase in the rate of acid-activation is thus explained. Treatment with dilute sea water should theoretically have the reverse effect, but in this case the experimental evidence is conflicting.

The acid-product *B* appears to be formed at a rate proportional (through a certain range) to the concentration of acid; it is regarded as being set free by the hydrolysis of some normal egg-component at a rate controlled by the pH. The special hypothesis that the hydrolyzed compound is a phosphagen ester has, however, received no support from test experiments.

The hypothesis that heat-activation is an effect of the formation of lactic acid by glycolysis is also not supported by experiments with iodoacetate and fluoride.

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